Molecular plasticity regulates oligomerization and cytotoxicity of the multipeptide-length amyloid-β peptide pool

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SUPPLEMENTARY INFORMATION

Experimental Procedures

Electrospray-Ionization Mass Spectrometry (ESI-MS) **-** Positive-ion mass spectra were recorded on an orthogonal acceleration quadrupole time-of-flight mass spectrometer (Q-Tof-2, Micromass, Manchester, U.K.) equipped with a standard electrospray probe (Z-spray) and controlled by a datasystem running MassLynx 3.4 (Micromass, Manchester, UK). Samples were dissolved in acetonitrile:water (1:1) containing 0.1 % acetic acid to a final concentration of 2 μM and infused using a syringe pump with a flow of 5 μl per minute. Cone voltage was set to 30 V, capillary voltage was 3 kV. Spectra were recorded from *m/z* 600 to *m/z* 1600. Scan time was set to 4.9 sec with an interscan time of 0.1 sec. At least ten spectra were acquired and averaged. Deconvolution was performed using the MaxEnt algorithm included in the software.

Molecular dynamics (MD) simulations - The structures of $A\beta_{1-38}$ and $A\beta_{1-40}$ were derived by excision of the last four (Val-Val-Ile-Ala) or the last two (Ile-Ala) residues of $A\beta_{1-42}$ (pdb 1IYT) respectively; while adding a threonine residue to the C-terminus of $A\beta_{1-42}$ generated $A\beta_{1-43}$. All MD simulations were performed with GROMACS 4.5.3, using the OPLS/AA force field (Jorgensen & Tirado-Rives, 1988). The N- and C-termini of each peptide were protonated and deprotonated, respectively, and all titratable amino acids were assigned their canonical state at physiological pH. The peptides were solvated in a triclinic box of explicit SPCE water model (Berendsen et al, 1987) with a minimum solute-box distance of 1.2 nm, to which 100 mM NaCl was added, including neutralizing counterions. Periodic boundary conditions were applied in all directions. Short-range nonbonded interactions were cut off at 1.0 nm, with long-range electrostatics calculated using the fourth-order (cubic) particle mesh Ewald (PME) algorithm (Darden et al, 1993; Essmann et al, 1995) with a Fourier-spacing of 0.16 nm for the FFT grid. Dispersion correction was applied to energy and pressure terms to account for truncation of van der Waals terms. Following steepest descents minimization (10,000 steps), each of the Aβ systems was equilibrated (Δt = 2 fs, leapfrog algorithm integrator) in two phases, with position restraints (1000 kJ mol^{-1} nm^{-2}) applied to peptide heavy atoms throughout. The first phase involved simulating for 100 ps under a constant volume (NVT) ensemble. Protein and nonprotein atoms were coupled to separate temperature-coupling baths, and temperature was maintained at 295 K using the velocity-rescale thermostat method (τ_T = 0.1 ps) (Bussi et al, 2007). Following NVT equilibration, 100 ps of constant-pressure (NPT) equilibration were performed, also using velocity-rescale method (τ_T = 0.1 ps) (Jorgensen & Tirado-Rives, 1988) for thermostating, and Parrinello-Rahman pressure coupler (τ_P = 2.0 ps, water compressibility of 4.5×10^{-5} bar^{-1}) (Parrinello & Rahman, 1981) to maintain pressure isotropically at 1.0 bar. Production MD simulations were conducted for 100 ns in the absence of any restraints, using the same thermostat and barostat methods used at the NPT equilibration phase.

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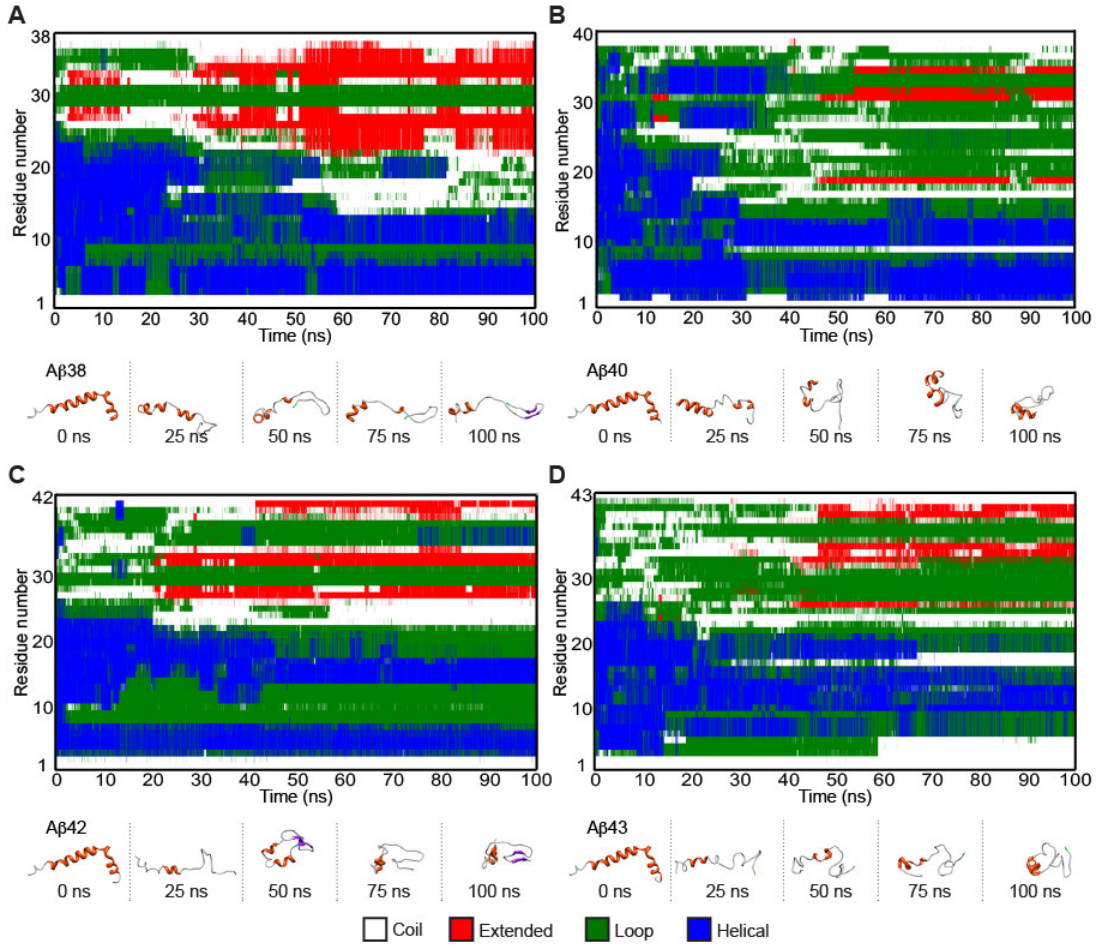
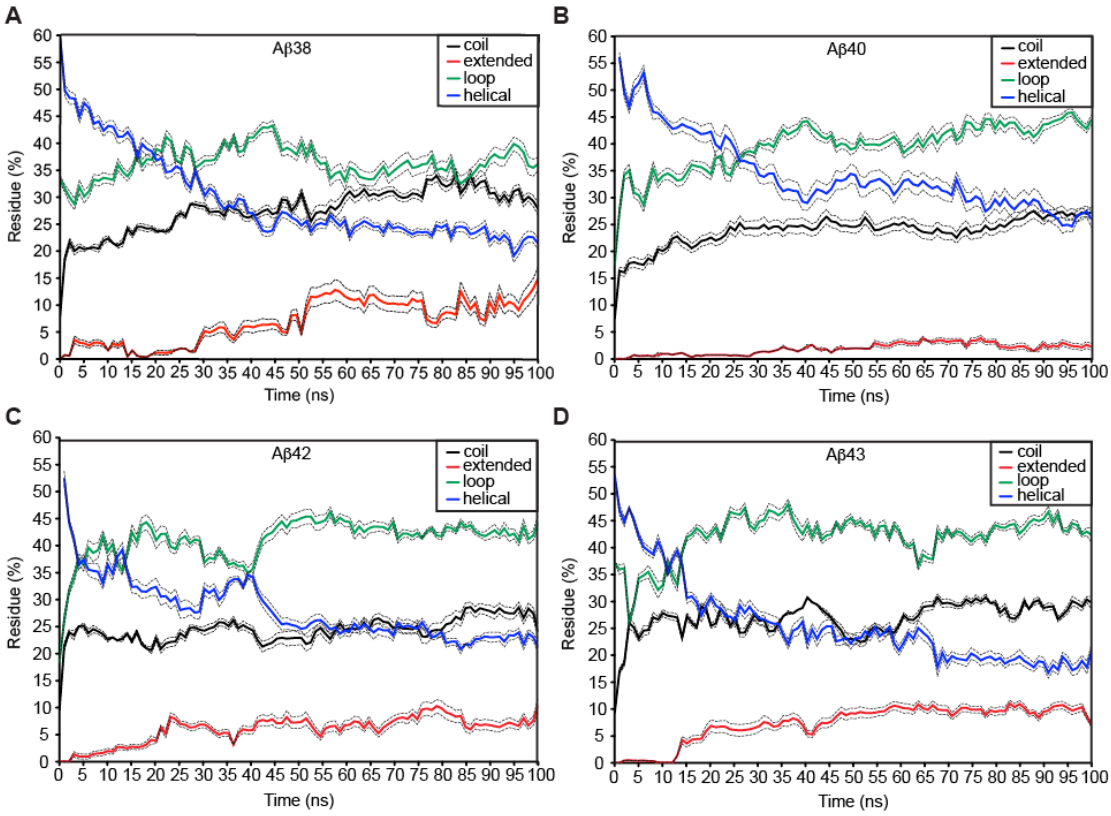
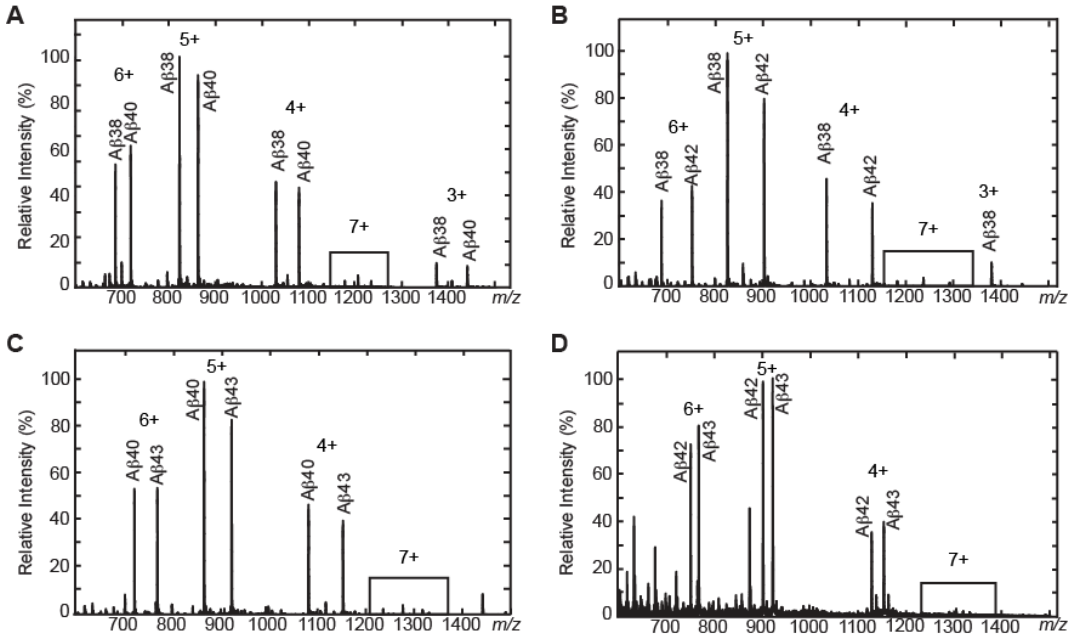


Supplementary figure 1: At short time scales extended conformations only occur at the C-termini of Aβ, while N-termini retain their helical conformation.
Secondary structure composition per residue in function of time for selected representative molecular dynamics simulations: (A) $A\beta_{1\text{-}38}$, (B) $A\beta_{1\text{-}40}$, (C) $A\beta_{1\text{-}42}$, and (D) $A\beta_{1\text{-}43}$. Secondary structure was determined using DSSP criteria. Reported conformations are: coil (unstructured conformation), extended conformation (β-bridge and β-sheet structures), loop (bend and turns), and helical conformation (α-helix, 3_{10}-helix, and π-helix). Snapshots of peptide conformations are shown for 0, 25, 50, 75 and 100 ns of simulation time.

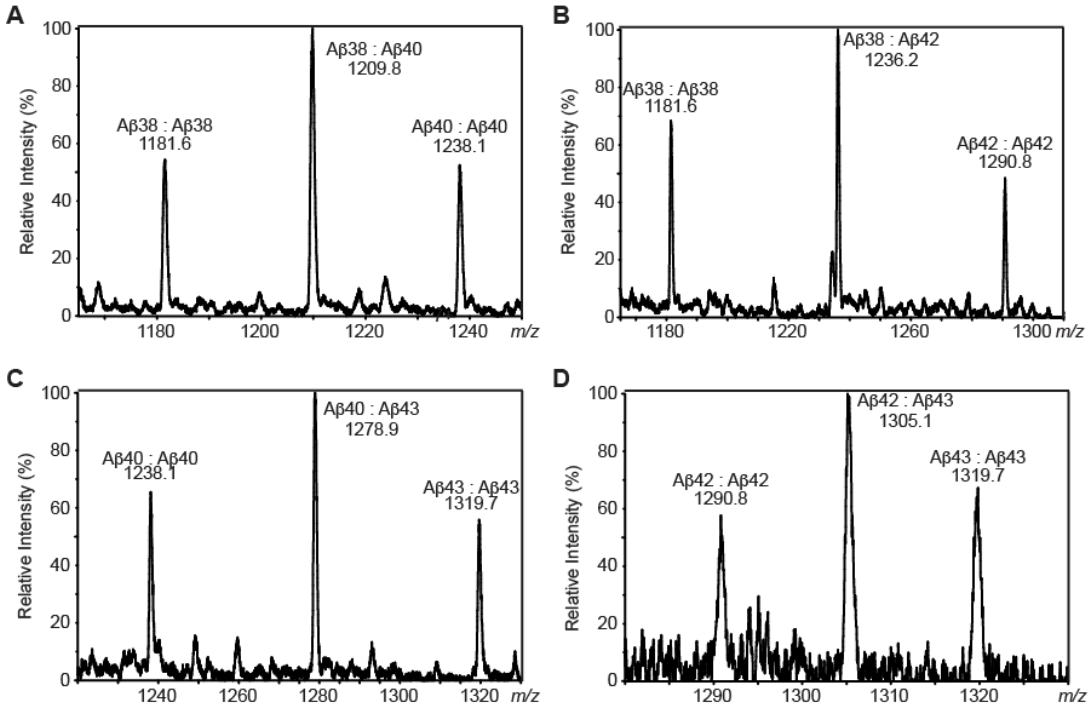


Supplementary figure 2: Secondary structure composition of $A\beta_{1\text{-}38}$ correlates better to $A\beta_{1\text{-}42}$ than to $A\beta_{1\text{-}40}$.
Overall appearance of secondary structure composition (% of total amount of residues) in function of time for (A) $A\beta_{1\text{-}38}$, (B) $A\beta_{1\text{-}40}$, (C) $A\beta_{1\text{-}42}$, and (D) $A\beta_{1\text{-}43}$. Secondary structure was determined using DSSP criteria, and reported as average of 10 simulations per peptide. Reported conformations correspond to: coil (unstructured conformation), extended conformation (β-bridge and β-sheet structures), loop (bend and turns), and helical conformation (α-helix, 3_{10}-helix and π-helix).



Supplementary figure 3: Aβ peptide mixtures show non-preferential random interaction.

Deconvoluted electrospray ionization mass spectra of 1:1 (A) $A\beta_{1\text{-}40}$:$A\beta_{1\text{-}38}$, (B) $A\beta_{1\text{-}42}$:$A\beta_{1\text{-}38}$, (C) $A\beta_{1\text{-}40}$:$A\beta_{1\text{-}43}$, and (D) $A\beta_{1\text{-}42}$:$A\beta_{1\text{-}43}$ were recorded immediately after mixing Aβ samples and 100-fold dilution in acetonitrile:water containing 0.1 % acetic acid. Spectra show intensity differences in peaks corresponding to both Aβ peptides indicating that mixtures were not exactly composed of 50 % of each peptide. Charge states of peaks are denoted on the graph. The region of the spectrum where dimers are detected is boxed. A zoom of this region is shown in Fig. S4.



Supplementary figure 4: Aβ peptide mixtures form small populations of mixed dimers.

Samples were mixed and diluted 100-fold in acetonitrile:water containing 0.1 % acetic acid directly before analysis by ESI-MS. Deconvoluted spectra of 1:1 (A) $A\beta_{1-40}$:$A\beta_{1-38}$, (B) $A\beta_{1-42}$:$A\beta_{1-38}$, (C) $A\beta_{1-40}$:$A\beta_{1-43}$, and (D) $A\beta_{1-42}$:$A\beta_{1-43}$ show interaction without preference to form homo- or heterodimers.